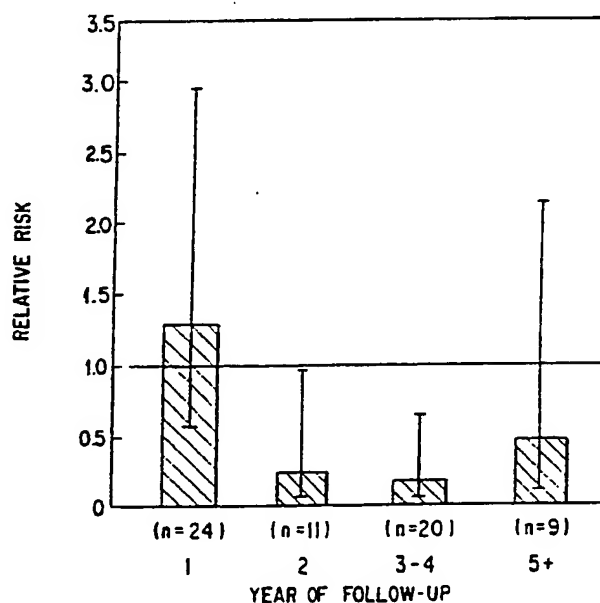




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: BETA-CAROTENE AND VITAMIN E THERAPY FOR INHIBITION OF MAJOR VASCULAR EVENTS



## (57) Abstract

This invention is directed to a method for inhibiting the occurrence of a major vascular event in a subject whereby a subject is administered beta-carotene and/or vitamin E, either alone or in combination. Beta-carotene and/or vitamin E is administered such that the subject's risk of experiencing a major vascular event is thereby reduced. In another preferred embodiment, beta-carotene in combination with aspirin is particularly effective. The invention further includes pharmaceutical compositions comprising beta-carotene in combination with vitamin E.

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## 10

## Field of the Invention

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### Brief Description of the Background Art

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a role in the pathogenesis of atherosclerotic disease (Steinberg et al., N. Engl. J. Med. 320(14):915-24 (1989)). Serum lipoproteins can become oxidized in vivo (Warso et al., J. Clin. Invest. 75:667-71 (1985)) and these may be more atherogenic than their unoxidized counterparts. Oxidized low density lipoprotein (LDL) can potentially promote atherogenesis by several mechanisms. First, these modified lipoproteins may be toxic to or alter function of arterial endothelium. Oxidized LDL is cytotoxic to cultured endothelial cells as well as fibroblasts in vitro (Hessler et al., Arteriosclerosis3(3):215-22 (1983); Yagi, K., Bioessays 1:58-60 (1984)). This altered endothelium may permit diffusion of serum lipids into the subendothelium and/or alter the ability of the endothelium to prevent thrombosis. Second, oxidized LDL chemotactically attracts and immobilizes monocyte/macrophages (Quinn et al., Proc. Natl. Acad. Sci. 82:5949-53 (1985)), some of which are destined to become lipid-laden foam cells within atheromatous plaque (Schaffner et al., Am. J. Pathol. 100:57-73 (1980); Gerrity, R.G., Am. J. Pathol. 103:181-90 (1981)). Finally, oxidized LDL is taken up into foam cells via a scavenger receptor more readily than unoxidized LDL (Fogelman et al., Proc. Natl. Acad. Sci. 77:2214-18 (1980); Goldstein et al., Proc. Natl. Acad. Sci. 76:333-37 (1979)). Thus oxidation of LDL may play an important role in the initiation and propagation of atherosclerosis.

Recent attention has focused on agents that may prevent the oxidation of LDL and thus impede or retard the progression of atherosclerosis. when incubated in various cell preparations, LDL will become oxidized (Morel et al., Arteriosclerosis4:357-64 (1984); Cathcort et al., J. Leukocyte Biol. 38:341-50 (1985)). This process can be inhibited by the addition to the cell preparation of antioxidants such as butylated hydroxytoluene or vitamin E (Morel et al., Arteriosclerosis4:357-64 (1984); Steinbrecher et al., Arteriosclerosis1:135-43 (1987)). LDL taken from patients treated with probucol, a cholesterol-lowering agent with lipid antioxidant properties, does not become oxidized in an endothelial

preparation that does oxidize LDL from untreated patients (Parthasarathy et al., J. Clin. Invest. 77:641-44 (1986)). This in vitro data suggests that antioxidant therapy may reduce the rate of in vivo lipid oxidation. In addition, after controlling for its lipid-lowering effect, probucol reduced the rate of fatty streak formation in Watanabe heritable hyperlipidemic rabbits (Carew et al., Proc. Natl. Acad. Sci. 84:7725-29 (1987)).

### Beta-Carotene

Beta-carotene is naturally occurring provitamin A with lipid antioxidant properties. In addition, beta-carotene is lipid soluble and is concentrated in circulating lipids and atherosclerotic plaques. Patients treated with a six week course of 160 mg of beta-carotene daily had a 50 fold increase in beta-carotene level within carotid plaques excised at the time of endarterectomy (Prince et al., Circulation 78:338-44 (1988)).

In vitro, beta-carotene is an unusual type of chain breaking lipid antioxidant (Burton et al., Science 224:569-73 (1984)). Because of its many conjugated double bonds, beta-carotene exhibits good radical trapping antioxidant behavior only at oxygen tension below 150 torr. Physiologic oxygen tension are typically below 100 torr.

There are few dietary studies on the association of beta-carotene to atherosclerotic disease. A cross-cultural study failed to show any association between serum beta-carotene levels and ischemic heart disease (Kok et al., Am. J. Clin. Nut. 45:462-68 (1987)).

Beta-carotene has virtually no important side effects. No biochemical or hematologic abnormalities were noted in 133 patients with erythropoietic protoporphyria treated with an average of 180 mg per day (doses as high as 300 mg per day) for as long as five years. The only reported side effect was skin discoloration, which is not evident at lower doses. In addition, one patient reported loose stools, which cleared

spontaneously (Mathews-Roth et al., Arch. Dermatol. 113:1299-1232 (1977)).

### Vitamin E

5 Vitamin E (alpha-tocopherol) is a fat soluble vitamin found in vegetable oils, egg yolk, milk fat, nuts, and cereal grains. Its primary function is felt to be as a lipid antioxidant protecting lipids from oxidative modification.

10 In vitro data confirm the ability of vitamin E to prevent the oxidation of lipids. During incubation with cultured endothelial cells, the LDL particle undergoes various structural changes that will alter its metabolism. These changes are dependent on lipid peroxidation as an initial step. This oxidative modification can be totally inhibited by the addition of vitamin E to the cellular preparation (Morel et al., Atherosclerosis4:357-64 (1984)).

15 Cross-cultural studies suggest an association between lipid standardized vitamin E levels and ischemic heart disease. In a large cross-cultural survey in which serum was collected from 903 randomly selected males aged 40-49 to determine the relationship of cholesterol-standardized vitamin E levels to rates of ischemic heart disease in six study populations (Gey et al., Am. J. Clin. Nutr. 45: 1368-77 (1987)), the vitamin E level  
20 within the plasma lipoproteins as indicated by the vitamin E/cholesterol ratio revealed significant inverse correlation to the ischemic heart disease mortality rate in each study population. In a nested case control study of Dutch men, no significant associations between serum vitamin E levels and cardiovascular disease mortality were noted (Kok et al., Am. J. Clin. Nutr. 45:462-68 (1987)).  
25 However, vitamin E levels were not lipid standardized. In animal studies, restricted ovulatory hens, which develop hyperlipidemia and subsequent aortic intimal thickening, were fed 1000 mg of vitamin E per Kg of feed (Smith et al., Atherosclerosis75:105-09

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(1989)). Compared to controls, those fed a vitamin E diet had reduced levels of plasma peroxides and less aortic intimal thickening. Variable results have been obtained from randomized clinical trials using vitamin E in various forms of atherosclerotic vascular disease. After 40 months of treatment with 600 mg of vitamin E daily in patients with claudication, 13 of 17 in the treatment group compared to only 2 of 17 in the placebo group showed improvement in symptoms (Livingston et al., Lancet 2:602-04 (1958)). These results have been confirmed in subsequent randomized trials (Haeger et al., Am. J. Clin. Nutr. 27:1179-81 (1974)). A randomized, placebo-controlled, double-blind, crossover study among patients with angina pectoris failed to show a benefit after six months of therapy with 1600 mg of vitamin E daily (Gillilan et al., Am. HeartJ. 93:444-49 (1977)).

Vitamin E is a safe drug with few clinically important side effects. Animal studies have shown that vitamin E is not carcinogenic or teratogenic (Federation of American Societies for Experimental Biology, Washington DC, (1975)). In human studies few side effects have been reported in double-blind protocols and other large studies, even at high doses (Anderson et al., Am. J. Clin. Nutr. 27:1174-78 (1974); Hole et al., J. Am. Diet. Assoc. 86:625-29 (1986)). Although oral intake of vitamin E at doses of 1200 mg/day may increase bleeding time in cardiac patients on warfarin (Corrigan et al., J.A.M.A. 230:1300-01 (1974)), vitamin E has not been shown to produce any coagulation abnormalities at lower doses among patients on warfarin or at any dose among individuals who are not vitamin K-deficient.

## SUMMARY OF THE INVENTION

We have discovered that the administration of Beta carotene and/or Vitamin E, either alone or in combination, to a subject, inhibits the occurrence of one or more major vascular events in the subject.

Major vascular events include, myocardial infarction, stroke, coronary revascularization procedure and cardiovascular death.

5 In particular, we have discovered that the occurrence of one or more major vascular events in a human subject that has experienced or is predisposed to experience angina pectoris, coronary artery bypass graft and/or percutaneous transluminal coronary angioplasty, is inhibited by the administration of beta-carotene and/or vitamin E to the subject.

10 This invention is further directed to a pharmaceutical composition comprising the synergistic combination of beta-carotene and vitamin E. The combination of beta-carotene and vitamin E is particularly useful in the therapeutic methods of the invention.

In yet a further embodiment, beta-carotene is administered in combination with aspirin.

### FIGURES

15 Figure 1 is a graphic representation of relative risk adjusted for age and aspirin assignment of major vascular events in the beta-carotene group, as compared with the placebo group, according to year of follow-up in the trial. Vertical bars represent confidence intervals. The number of events for each interval are listed below each bar.

20 Figure 1 also shows that the effect of beta-carotene is chronic, not acute, and that beta-carotene delays the progression of atherosclerosis.

### DESCRIPTION OF THE PREFERRED EMEODIMENTS

25 A preferred method of the invention includes the administration of beta-carotene and/or vitamin E to a subject such that the occurrence of one or more major vascular events is thereby inhibited.

Another preferred method of the invention includes the administration of a composition comprised of the synergistic combination

of beta-carotene and vitamin E to a subject such that the occurrence of one or more major vascular events is thereby reduced. Included as well in the present invention are pharmaceutical compositions comprised of beta-carotene and vitamin E in combination with a pharmaceutically acceptable carrier.

More specifically, by the term "inhibiting" is intended both prevention and amelioration of the disease state. Thus the invention comprises both a therapeutic and a prophylactic modality.

By the term "major vascular events" is generally intended atherosclerotic vascular disease and its related illnesses. In particular, major vascular events includes myocardial infarction, stroke, coronary revascularization procedure and cardiovascular death.

By the term "beta-carotene" is intended beta-carotene and the biologically active analogs thereof. Typical analogs include molecules which demonstrate equivalent biological function but which differ structurally. Such analogs include cantaxan thin, astaxanthin, zeaxantin, luetin and lycopin.

By the term "vitamin E" is intended vitamin E and the biologically active analogs thereof. Typical analogs include molecules which demonstrate equivalent biological function but which differ structurally. Such analogs include all other tocopherols.

By the term "aspirin" is intended aspirin and the biologically active fractions thereof.

By the term "a composition of beta-carotene in combination with vitamin E" is intended any composition comprising beta-carotene or beta-carotene analogs, and vitamin E or analogs of vitamin E.

By the term "a composition of beta-carotene in combination with aspirin" is intended any composition comprising beta-carotene or beta-carotene analogs, and aspirin or analogs of aspirin.

By the term "subject" is intended all mammals, in particular humans.



By the term "administration" is intended the administration of the pharmaceutical compositions of the present invention by any means that achieve their intended purpose. For example, administration may be by parenteral, subcutaneous, intravenous, intramuscular, intra-peritoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

Compositions within the scope of this invention include all compositions of 500 mg or less of beta-carotene, in combination with 5000 mg or less of vitamin E; and all compositions of 500 mg or less of beta-carotene, in combination with 2400 mg or less of aspirin, in an amount which is effective to achieve its intended purpose. While individual needs vary, determination of optimal ranges of effective amounts of each component is with the skill of the art. Typically, beta-carotene and/or vitamin E may be administered to mammals, e.g. humans, orally at a dose of 25-300 mg per day of beta carotene and 100-3200 mg per day of vitamin E, or an equivalent amount of beta-carotene, vitamin E and the beta-carotene/vitamin E composition, per day of the body weight of the mammal being treated for major vascular disorders. Preferably, about 50 mg of beta-carotene and/or 600 mg of vitamin E are orally administered to treat or prevent such disorders.

The unit oral dose may comprise from about 0.25 to about 500 mg, preferably about 3 to about 100 mg of beta-carotene; about 5 to about 5000 mg, preferably about 100 to about 1000 mg of vitamin and about the same relative amounts of each for the composition combining beta-carotene and vitamin E. The unit dose may be administered one or more times daily or on alternate days.

In addition to administering beta-carotene and/or vitamin E as a raw chemical, the compounds may be administered as part of a pharma-

5        ceutical preparation containing suitable pharmaceutically acceptable  
carriers, excipients and auxiliaries which facilitate processing of beta-  
carotene and/or vitamin E into preparations which can be used  
pharmaceutically. Preferably, the preparations, particularly those  
10       preparations which can be administered orally and which can be used for  
the preferred type of administration, such as tablets, dragees, and  
capsules, and also preparations which can be administered rectally, such  
as suppositories, as well as suitable solutions for administration by  
injection or orally, in dose ranges that provide similar bioavailability as  
15       described above, together with the excipient.

      The pharmaceutical preparations of the present invention are  
manufactured in a manner which is itself known, for example, by means  
of conventional mixing, granulating, dragee-making, dissolving, or  
lyophilizing processes. Thus, pharmaceutical preparations for oral use can  
15       be obtained by combining the active compounds with solid excipients,  
optionally grinding the resulting mixture and processing the mixture of  
granules, after adding suitable auxiliaries, if desired or necessary, to obtain  
tablets or dragee cores.

      Suitable excipients are, in particular, fillers such as saccharides, for  
20       example lactose or sucrose, mannitol or sorbitol, cellulose preparations  
and/or calcium phosphates, for example tricalcium phosphate or calcium  
hydrogen phosphate, as well as binders such as starch paste, using, for  
example, maize starch, wheat starch, rice starch, potato starch, gelatin,  
tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium  
25       carboxymethylcellulose, and/or polyvinyl pyrrolidone. If desired,  
disintegrating agents may be added such as the above-mentioned starches  
and also carboxymethyl-starch, cross-linked polyvinyl pyrrolidone, agar, or  
alginic acid or a salt thereof, such as sodium alginate. Auxiliaries are,  
above all, flow-regulating agents and lubricants, for example, silica, talc,  
30       steric acid or salts thereof, such as magnesium stearate or calcium  
stearate, and/or polyethylene glycol. Dragee cores are provided with

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5 suitable coatings which, if desired, are resistant to gastric juices. For this purpose, concentrated saccharide solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, polyethylene glycol and/or titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. In order to produce coatings resistant to gastric juices, solutions of suitable cellulose preparations such as acetylcellulose phthalate or hydroxypropymethyl-cellulose phthalate, are used. Dye stuffs or pigments may be added to the tablets or dragee coatings, for example, for identification or in order to characterize combinations of active compound doses.

10 Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, or liquid paraffin. In addition, stabilizers may be added.

20 Possible pharmaceutical preparations which can be used rectally include, for example, suppositories, which consist of a combination of the active compounds with a suppository base. Suitable suppository bases are, for example, natural or synthetic triglycerides, or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the active compounds with a base. Possible base materials include, for example, liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

25 Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable

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lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

The entire text of all applications, patents and publications, if any, cited above and below are hereby incorporated by reference.

### EXAMPLES

Having now described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

#### Example 1

A study was conducted in which a randomized trial of aspirin was tested for its effect on the reduction of cardiovascular mortality; and beta-carotene (provided as LUROTIN™ by BASF) was simultaneously tested for its effect on the reduction of cancer incidence. The trial was conducted entirely by mail among 22,071 male physicians, aged 40 to 84, who were randomized to take either a 325 mg aspirin or placebo tablet every other day alternating with a capsule containing 50 mg beta-carotene or placebo. The trial thus used a 2x2 factorial design, with each participant taking one pill daily (Stampfer et al., Stat. Med. 4:111-16 (1985)).

The 261,248 potentially eligible physicians, identified from an age-targeted list provided by the American Medical Association, were sent letters of introduction, consent forms and enrollment questionnaires. A total of 33,223 doctors enrolled in an 18-week run-in phase. Participants

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took daily pills from calendar packs containing active aspirin alternating with beta-carotene placebo. This run-in period enabled the identification and elimination of poor compliers and those who could not tolerate aspirin before randomization, thus increasing the power of the study. At the end of the run-in, a total of 22,071 (66%) were randomized to aspirin, beta-carotene, both or neither. Use of the run-in strategy appears to have been quite effective. At 60.2 months of follow-up, (averaged for participants, ranging from 45.8 to 77.0) over 90% of all participants reported taking 50% or more of their study pills for the entire duration of the trial, 86.7% were still taking the beta-carotene/placebo capsules, and 84% were still taking the aspirin/placebo tablets. Not a single participant has been lost with respect to mortality follow-up, and 99.7% are still providing information on morbidity. The blinded aspirin component of the study was terminated prematurely due primarily to the emergence of a statistically extreme benefit of aspirin on both fatal and nonfatal MS (The Steering Committee of the Physicians' Health Study Research Group, N. Engl. J. Med. 318:262-64 (1988)), while the beta-carotene component is still ongoing.

Of particular relevance to the present invention are the studies concerning beta-carotene's role in cardiovascular disease. These studies were conducted among the subgroup of participants who entered the trial with a history of angina pectoris or coronary revascularization (coronary artery bypass surgery or percutaneous transluminal angioplasty), and indicated a marked and statistically significant benefit of beta-carotene in reducing subsequent vascular events among this high-risk population.

The results, as set forth in Table 1, indicate that the reduction of risk for patients taking beta-carotene, as compared to placebo, did not occur until beta-carotene had been taken for a long period of time.

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Table 1

Cardiovascular End Points in Patients with  
Prerandomization Angina Pectoris and/or Coronary Revascularization  
According to Beta Carotene Assignment\*

	Beta Carotene (n=160)	Placebo (n=173)	Relative Risk**	95% Confidence Interval	P Value
Revascularization (Revasc.)***	10	21	0.43	0.17-1.11	0.08
Myocardial Infarction (MI)	10	17	0.61	0.31-1.40	0.29
Stroke	4	9	0.47	0.15-1.55	0.22
Cardiovascular Death (CV Death)	7	6	1.29	0.42-4.01	0.66
Total Death	8	10	0.85	0.29-2.51	0.77
Major coronary event (Revasc., MI, Coronary Death)	20	36	0.56	0.31-0.99	0.047
Major vascular event (Major coronary event, Stroke)	22	42	0.51	0.30-0.88	0.015

\*End Points included only the first event within each category.

\*\*Adjusted for age and aspirin assignment.

\*\*\*Defined as coronary artery bypass graft or percutaneous transluminal angioplasty.

CLAIMSWHAT IS CLAIMED IS:

1. A method for inhibiting the occurrence of a major vascular event in a subject comprising administering beta-carotene or vitamin E to said subject.  
5
2. The method of claim 1, wherein said major vascular event is selected from the group consisting of myocardial infarction, stroke, coronary revascularization procedure and cardiovascular death.
3. The method of claim 1, wherein said beta-carotene or vitamin E is administered to said subject subcutaneously.  
10
4. The method of claim 1, wherein said beta-carotene or vitamin E is administered to said subject orally.
5. The method of claim 1, wherein said beta-carotene is administered to said subject in an amount ranging from 0.25 mg to 500 mg on alternate days or daily.  
15
6. The method of claim 1, wherein said vitamin E is administered to said subject in an amount ranging from 5 mg to 5000 mg on alternate days or daily.
7. The method of claim 1, wherein said subject is a human subject that has experienced or is predisposed to experience angina pectoris and/or coronary revascularization.  
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8. The method of claim 7, wherein said coronary revascularization is selected from the group consisting of coronary artery bypass graft or percutaneous transluminal coronary angioplasty.

5 9. A method for preventing the occurrence of a major vascular event in a subject comprising administering a composition of beta-carotene in combination with vitamin E.

10. The method of claim 9, wherein said major vascular event is selected from the group consisting of myocardial infarction, stroke, coronary revascularization procedure and cardiovascular death.

10 11. The method of claim 9, wherein said composition of beta-carotene and vitamin E is administered to said subject subcutaneously.

12. The method of claim 9, wherein said composition of beta-carotene and vitamin E is administered to said subject orally.

15 13. The method of claim 9, wherein said composition of beta-carotene and vitamin E is administered to said subject in an amount ranging from 0.25 mg to 500 mg of beta-carotene and 5 mg to 5000 mg of vitamin E on alternate days or daily.

20 14. The method of claim 9, wherein said subject at risk is a human subject that has experienced or is predisposed to experience angina pectoris and/or coronary revascularization.

15. The method of claim 14, wherein said coronary revascularization may be either one or more coronary artery bypass graft(s), or one or more percutaneous transluminal coronary angioplasty(s).



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16. A pharmaceutical composition suitable for inhibiting a major vascular event in a subject, said composition comprising beta-carotene and vitamin E.

5 17. A method for preventing the occurrence of a major vascular event in a subject comprising administering a composition of beta-carotene in combination with aspirin.

18. The method of claim 17, wherein said major vascular event is selected from the group consisting of myocardial infarction, stroke, coronary revascularization procedure and cardiovascular death.

10 19. The method of claim 17, wherein said composition of beta-carotene and aspirin is administered to said subject subcutaneously.

20. The method of claim 17, wherein said composition of beta-carotene and aspirin is administered to said subject orally.

15 21. The method of claim 17, wherein said composition of beta-carotene and aspirin is administered to said subject in an amount ranging from 0.25 mg to 500 mg of beta-carotene and 5 mg to 2400 mg of aspirin on alternate days or daily.

20 22. The method of claim 17, wherein said subject at risk is a human subject that has experienced or is predisposed to experience angina pectoris and/or coronary revascularization.

23. The method of claim 22, wherein said coronary revascularization may be either one or more coronary artery bypass graft(s), or one or more percutaneous transluminal coronary angioplasty(s).

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US91/07093

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) \*

According to International Patent Classification (IPC) or to both National Classification and IPC  
 IPC(5): A61K 31/07, 31/355, 31/605  
 U.S. CL. 424/422

## II. FIELDS SEARCHED

Minimum Documentation Searched <sup>7</sup>

Classification System	Classification Symbols
U.S.	424/422; 514/458, 165, 558

Documentation Searched other than Minimum Documentation  
 to the Extent that such Documents are included in the Fields Searched <sup>8</sup>

## III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup>

Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
Y	BURTON ET. AL., "β-Carotene: An Unusual Type of Lipid Antioxidant" <u>Science</u> , Volume 224, 11 MAY 1984, See pages 569-573.	1-24
Y	PARTHASARATHY ET. AL. "Probucol Inhibits Oxidative Modification of Low Density Lipoproteins", <u>J. Clinical Invest.</u> , Volume 77, FEBRUARY 1986, See pages 641-644.	1-24
Y	STEINBERG ET. AL., "Modifications of Low-Density Lipoproteins That Increase Its Atherogenicity", <u>New England Journal of Medicine</u> , Volume 320, No. 14, 06 APRIL 1989. See pages 915-924.	1-24

\* Special categories of cited documents: <sup>10</sup>

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

## IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
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11 December 1991

13 JAN 1992

International Searching Authority

Signature of Authorized Officer

ISA/US

*Thurman K. Page Jr*  
 David J. Colucci